

Appl. No. : 10/796,298
Filed : March 9, 2004

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0078] of the specification with the following paragraph:

[0078] Preferred embodiments of the lysis buffer include 10 to $1e^{10}$, more preferably $1e^5$ to $1e^{10}$, copies of spiked RNA per well. In preferred embodiments, the amount of control RNA used is at least enough to be detected, but not so much as to significantly interfere with the amount of target mRNA that is quantified. In preferred embodiments, the control RNA added to the lysis buffer is poly(A)⁺ RNA. In particularly preferred embodiments where the sample being tested is human blood, the control RNA is not homologous to RNA present in human blood. In some preferred embodiments, the sequence of the control RNA is less than 90% homologous to the target mRNA, or has greater than 10% difference in length with the target mRNA. In other preferred embodiments, the sequence of the control RNA is less than 85% homologous to the target mRNA, or has greater than 5% difference in length with the target mRNA. In further embodiments, the sequence of the control RNA is less than 75% homologous to the target mRNA, or has greater than 2% difference in length with the target mRNA. In alternative embodiments, the sequence of the control RNA is less than 65% homologous to the target mRNA, or has greater than 1% difference in length with the target mRNA. In one embodiment, control RNA may preferably be made by amplifying template oligonucleotides by means of PCR. Thus, forward primers (SEQ ID NOs 2510, 2611, 7515, and 238), reverse primers (SEQ ID NOs 249, 7616, and 249), and TaqMan probes (FAM-SEQ ID NOs 2813-TAMRA, FAM-SEQ ID NO 7717-TAMRA, and FAM-SEQ ID NO 2712-TAMRA) can be used to amplify various control RNA oligonucleotides. Alternative embodiments comprise using a plurality of different target mRNAs to be quantified. Further embodiments comprise using a plurality of control RNAs.

Please replace paragraphs [0100] through [0107] of the specification with the following paragraphs:

[0100] **Preparation of control RNA.** In order to synthesize control RNA, template oligonucleotides (SEQ ID NOs 34-2 and 364) and cDNA from K562 cells

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(RNA_{ture}, Irvine, CA) were amplified with T7-forward primers (SEQ ID NOs 353, 375, and 746) and dT₄₀ reverse primers (T₄₀-SEQ ID NOs 341, and T₄₀-SEQ ID NO 767) with 30 cycles of 95°C denaturing for 30 sec, 55°C annealing for 10 sec, followed by 72°C extension for 20 sec, respectively. Oligonucleotides were purchased from IDT (Coralville, IA) or Proligo (Boulder, CO). The sequences were as followsed:

[0101] SEQ ID NO 341: 5'-~~T₄₀~~-GGGTG CTGTG CTTCT GTGAA C-3',

[0102] SEQ ID NO 342: 5'-GCCCC CTCAC TCCCA AATTC CAAGG CCCAG CCCTC ACACA TTGTT CACAG AAGCA CAGCA CCC-3',

[0103] SEQ ID NO 353: 5'-GTAAT ACGAC TCACT ATAGG GGGAC AGCCC CCTCA CTCCC AAA-3',

[0104] SEQ ID NO 364: 5'-GAAGC GTGTG TCACT GTGTG TTTCC AAGGC CCAGC CCTCA CACAT TGTTC ACAGA AGCAC AGCAC CC-3',

[0105] SEQ ID NO 375: 5'-GTAAT ACGAC TCACT ATAGG GGGAC GGAAG CGTGT GTCAC TGTGT GT-3',

[0106] SEQ ID NO ~~74~~T76: 5'-GTAAT ACGAC TCACT ATAGG GGGAC GCATT CCGCT GACCA TCAAT A-3',

[0107] SEQ ID NO ~~76~~T407: ~~T₄₀~~5'-TCCAA CGAGC GGCTT CAC-3'.

Please replace paragraphs [0112] through [0122] of the specification with the following paragraphs:

[0112] **TaqMan real time PCR.** Primers and TaqMan probes for control RNA were designed by Primer Express version 2.0 (ABI, Foster City, CA). For *bcr-abl*, we used published sequences. In some experiments, HYBsimulator (RNA_{ture}) was used to design reverse primers. The forward primers (SEQ ID NOs 2510, 2611, 7515, and 238), reverse primers (SEQ ID NOs 249, and 7616), and TaqMan probes (FAM-SEQ ID NOs 2813-TAMRA, FAM-SEQ ID NO 7717-TAMRA, and FAM-SEQ ID NO 2712-TAMRA) were used to amplify control RNA. In order to determine the amounts of CD4 mRNA in blood samples, both CD4 and control RNA were analyzed in the different wells of PCR plates, rather than multiplex PCR in a single well. For β -actin, commercially available primers and probes were used (ABI). Into a 384 well PCR plate (ABI) were mixed: 2 μ L of cDNA, 5 μ L of TaqMan universal master mix (ABI), 1 μ L of 5 μ M of forward primers, 1 μ L of 5 μ M of

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reverse primers, and 1 μ L of 2 μ M TaqMan probe. PCR was conducted in an ABI PRISM 7900HT (ABI), using 1 cycle of 95°C for 10 minutes, followed by 45 cycles of 95°C for 20 seconds, followed by 55°C for 20 seconds, and finally 60°C for 1 minute. The data were analyzed by SDS version 2.0 (ABI). In some experiments, the TaqMan assay was conducted directly in a GenePlate (Opticon, MJ Research). Oligonucleotides (SEQ ID NOs 342, 364, and 2914) and PCR products were used as quantitation standards for control RNA. The sequences were as follows:

- [0113] SEQ ID NO 238: 5'-AAATG CCACA CGGCT CTCA -3'
- [0114] SEQ ID NO 249: 5'-CAAGT GTCTT CGTGT CGTGG G-3'
- [0115] SEQ ID NO 2510: 5'-AGCCC CCTCA CTCCC AAA-3'
- [0116] SEQ ID NO 2611: 5'-AGCCC CCTCA CTCCC AAA-3'
- [0117] SEQ ID NO 2712: 5'-~~FAM~~-CAGTG GCTAG TGGTG GGTAC TCAAT GTGTA CTT-~~TAMRA~~-3'
- [0118] SEQ ID NO 2813: 5'-~~FAM~~-CCAAG GCCCA GCCCT CACAC A-~~TAMRA~~-3'
- [0119] SEQ ID NO 2914: 5'- CAGG GACAA ATGCC ACACG GCTCT CACCA GTGGC TAGTG GTGGG TACTC AATGT GTACT TTTGG GTTCA CAGAA GCACA GCACC CAGGG-3',
- [0120] SEQ ID NO 7515: 5'-CCACT GGATT TAAGC AGAGT TCAA-3'
- [0121] SEQ ID NO 7616: 5'-TCCAA CGAGC GGCTT CAC-3'
- [0122] SEQ ID NO 7717: 5'-~~FAM~~-CAGCG GCCAG TAGCA TCTGA CTTTG A-~~TAMRA~~-3'